Comprehensive morphological and molecular analyses of flowering events in *Bambusa tulda*

Summary of work

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1. Summary of work: Comprehensive morphological and molecular analyses of flowering events in *Bambusa tulda.*

2. Introduction

Flowering is one of the most important adaptations in the history of plant evolution. At present, most of the studies to understand the regulation of flowering time have been conducted on the model plants *Arabidopsis thaliana* and *Oryza sativa*. Since both of these plants are annual and herbaceous, it is unlikely that the genetic model developed from these studies can directly be translated to explain the uncertain flowering behaviour in perennial, monocarpic trees like bamboo. Bamboo plants possess a unique flowering behaviour, which may vary from 3 to 120 years depending on the species (Janzen, 1976). Based on the flowering behaviour bamboos have been grouped in three major categories- annual flowering, sporadic/irregular flowering and mass/gregarious flowering (Brandis, 1899). In the event of gregarious flowering, all the bamboo plants of a particular species flower over a large stretch of geographical area and then all of them die together. The sudden disappearance of such large vegetation in a forest can causes major ecological and also economic disasters. In many states of India it is regarded as a bad omen due to substantial amount of crop loss lead by an increase of rodent's population soon after flowering and seed setting of bamboo (Azad-Thakur et al., 2014). Many of these ecological impacts caused due to bamboo flowering are known for decades, but our knowledge about reproductive biology and also the genes, small RNAs and metabolites controlling such unique flowering behaviour is inadequate.

Due to gross unavailability, very limited studies could have been done to study the individual inflorescence and floral organ characteristics features in different bamboo species such as *Bambusa tulda* (Das et al., 2005), *Bambusa vulgaris* (Koshy and Jee, 2001), *Dendrocalamus hamiltonii* (Cavendish, 1895), *D. longispathus* (Mohan Ram and Hari Gopal, 1981) and a few more. However, no in depth studies have been conducted to study the detailed reproductive features of bamboos such as developmental progression patterns of individual floral organs, pollen germination, pollen-pistil interaction and seed setting. Therefore, a major objective of this study is understand the detailed reproductive features of bamboo using *Bambusa tulda* as the reference plants since i) it is widely growing in West Bengal ii) commercially important and iii) flowering incidents in this species could have been obtained during the survey of bamboo population in and around West Bengal.

Molecular understanding on the induction of bamboo flowering is also severely lacking (Biswas et al., 2016). Only a limited number of studies have been conducted either to identify flowering gene homologs from diverse bamboos species (Bo et al., 2005; Hisamoto et al., 2008; Zeng et al., 2015; Liu et al., 2015; Lin et al., 2009; Wang et al., 2014 ; Shih et al., 2014) or sequencing of ESTs from floral tissue specific transcriptome libraries (Lin et al., 2010; Liu et al., 2012; Zhang et al., 2012; Peng et al., 2013; Gao et al., 2014; Ge et al., 2016; Wysocki et al., 2016; Zhao et al.,

2016). A limited number of flowering genes in different bamboo species such as MADS18 from *Dendrocalamus latiflorus* (Bo et al., 2005), FLOWERING LOCUS T (FT) from *P.*

meyeri (Hisamoto et al., 2008), TERMINAL FLOWER 1 (TFL1) like gene from *Bambusa oldhamii* (Zeng et al., 2015), FRIGIDA (FRI) from *P. violascens* (Liu et al., 2015), MADS1 and MADS2 from P. praecox (Lin et al., 2009), 10 genes related to floral transition and meristem identity in *D. latiflorus* (Wang et al., 2014) and 16 MADS box genes from *B. edulis* (Shih et al., 2014).

However, no targeted and systematic studies have been undertaken to characterize these gene sequences and their expression characterization to understand the role in bamboo flower induction.

3. Objective of the study

On the basis of the above stated discussions on the current status of understanding of the topic, the major objectives of this Ph. D. dissertation studies are as follows:

Objective 1. Identification of rare flowering features of *Bambusa tulda* .
I. Detailed morphological characterization of *B. tulda* inflorescence and floral characters
II. Histological analyses of floral developmental progression.

Objective 2. Morphological and biochemical analyses of pollen to understand the pollen viability
I. Anther and pistil development in *B. tulda*
II. Studies on pollen viability with staining and *in-vitro* germination methods

Objective 3. Isolation and molecular characterization of *FLOWERING LOCUS T* (*FT*) gene/s and regulator genes of *FT*, if any, from *B. tulda*.
I. Bioinformatics identification of flowering gene homologs in temperate bamboo *Phyllostachys edulis*
II. Isolation, molecular characterization and tissue specific expression analyses of *FLOWERING LOCUS T* (*FT*) gene homologs.
III. Isolation and molecular characterization and tissue specific expression analyses of *FD* gene homologs

4. Methodology

I. Sample collection and study on the *B. tulda* specific morphological characters across populations

Extensive field survey has been done for morphological studies and also to identify flowering events in bamboos. Areas of field survey includes district of Darjeeling, North 24 PGS, Hoogly, Burdwan, Nadia and Medinipur East. Sporadic flowering events were recorded in two different populations located at Shyamnagar (SHYM7, SHYM16) and in a population located at Bandel (BNDL22), Habra (HAB1), and Behula (BEH4). Seventeen key culm sheath (Table 1) and fifteen culm characters (Table 2) were recorded for morphological

comparison with other bamboo species and also among populations. The studied culm sheath characters included size of culm sheath, ratio of total length to breadth at base, margin of sheath (ciliate or not), adaxial or abaxial surface (pubescent or not), number of hairs (absent, scanty or profuse), and hair colour (none, golden brown, brown, dark brown or black). The characters of blade studied were ratio of total length versus blade length, blade shape (triangular, acuminate, lanceolate or ovate), alignment and character of the margin. The ligule characters studied were types of ligule margin (entire, dentate or serrate), ligule hairs, nature of auricle (auricle continuous with blade or not) shape of auricle and frequency of bristles present. The important culm characters studied were mean height (m), diameter (mm), length of fifth internode (mm) and ratio of cavity diameter to total culm diameter. Other culm characters included bending of internode, culm colour (yellow with striation, yellow green, gray green, pale green, bright green, glossy green or dark green), nature of node (swollen or not), nature of nodal ring (absent, whitish or greyish), nodal sheath scar and presence or absence of hairs at nodal ring., Difference in culm leaf and branch leaf size, any modification of branches (none, thorns or spines) and striation on culm were also recorded as key identifying characters. Together all of these important thirty two morphological characters of *B. tulda* obtained from 219 populations were compared with twenty five other bamboo species reported in Das et al. (2008). Voucher specimens (deposition nos.-56A, 56B, 57A, 57B, 58A. 58B, 59A, 59B, 59C dated 05.06.2015) were submitted to Acharya Jagadish Chandra Bose Indian Botanic Garden, Shibpur, Howrah.

II. Amplification of species-specific SCAR marker to confirm *B. tulda* species identity

The identity of *B. tulda* species was further confirmed by obtaining PCR amplification of $Tuldo_{600}$ SCAR marker, which is specific to *B. tulda* (Das et al., 2005).

III. Morphological characterization of *B. tulda* inflorescence and floral characters

Fresh floral tissue samples were collected from three different flowering *B. tulda* populations (SHYM7, SHYM16, BNDL22) and the flower characters such as number of spikelet per head, number of florets per spikelet, morphological characters of individual florets and dissected floral parts were studied under stereo zoom microscope (Carl Zeiss, Germany).

IV. Histological analyses to study developmental progression of *B. tulda* florets

To study the developmental progression of *B. tulda* florets and floral organs, histology of early, middle and late developmental stages of spikelets were studied by longitudinal section. The sections were dehydrated by exposure to alcohol solutions of gradually increasing concentrations (50, 70, 95 and 100%). Double staining was done treating with 1% safranin after dehydration with 50% alcohol and with 0.5% light green solutions after dehydration with 95% alcohol. Finally the samples were washed twice with absolute alcohol and then with xylene before observing them under light microscope (Carl Zeiss, Axiostar Plus). Images were captured with 'Carl Zeiss MicroImaging GmbH 37081' imaging system.

IV. Morphological and biochemical analyses of *B. tulda* pollen

To study the pollen morphology and viability of *B. tulda*, pollens from mature anther collected from different populations were collected in 1.5 ml micro centrifuge tube. Three different staining techniques were used to assess pollen viability.

(a) Acetocarmine test: The pollen grains were stained with 1: 1 mixture of glycerine and 2% acetocarmine and observed under a microscope. Fertile pollens were stained in red where as the unstained ones was considered as sterile (Koshy and Jee, 2001).

(b) I_2-KI test: Iodine solution was prepared by dissolving 8g potassium iodide (KI) and 1g iodine (I_2) in 10 ml distilled water (Kearns and Inouye, 1993). Pollens were treated with the iodine solution and observed under microscope. Black-stained pollen was considered as viable, while unstained pollens were non-viable.

(c) Tetrazolium test: Freshly collected mature pollen grains were dusted in a drop of 0.5% TTC (2,3,5 triphenyltetrazolium chloride) in sucrose solution (15%) and incubated in a 1.5 ml micro centrifuge tube at room temperature in dark for 30 mins following established protocols described before (Hauser and Morrison, 1964, Stanley and Linsken, 1974). Then they were spread over a slide and observed under the microscope. The pollen grains, which were stained red, were counted as viable and the unstained ones were considered as sterile.
Pollens collected from each of the three studied populations were studied and data from three random microscopic fields were collected to calculate the frequencies of viable and nonviable pollen grains. The pollen grains that were round shaped and properly stained were scored as fertile, while those that were compressed and unstained were scored as sterile. Percentage of pollen viability was calculated by dividing the number of stained pollens by the total number of pollen grains per field of view and expressed in percentage values. Pollens of *O. sativa* cv. IR64 were used as control.

V. *In vitro* pollen germination assay

In vitro pollen germination studies were carried out by using freshly collected pollen grains dusted in standard Brewbaker and Kwack's medium on a slide (Heslop-Harrison and Heslop-Harrison, 1970), supplemented with 5, 10, 15, 20, 25, 30 and 35% sucrose and were incubated for 30 mins before microscopic observation . Pollen grains were considered as germinated if the length of pollen tube was greater than the diameter of the pollen grain as per the recommended procedure (Tuinstra and Wedel, 2000). Germination percentage was determined by dividing the number of germinated pollen grains per field of view by the total number of pollen per field.

VI. Bioinformatics identification of flowering gene homologs in temperate, sequenced bamboo *Phyllostachys edulis*

Oryza sativa is the reference monocot plant that has extensively been studied to characterize all major flowering pathways such as photoperiodic, vernalization, hormonal, and autonomous (Higgins et al., 2011). In addition some studies have also been conducted on the phylogenetically close monocot species *Brachypodium distachyon* (Higgins et al., 2011). In order to identify the bamboo flowering genes homologous, the only sequenced draft bamboo

genome *P. edulis* was queried by using rice or *Brachypodium* genes as query. In addition to established pathway genes, the role of additional factors such as drought, stress and physiological maturity have been tested by targeting appropriate marker genes. The locus Id of the forty key flower inducing rice genes were obtained either by extensive literature search or from genome databases (ref.). Only those locus Ids were used for which the functional identity of the genes have been confirmed by loss/gain of function mutational analyses. If data was not available for *O. sativa* then sequence from *B. distachyon* were used for this analyses. The fixed criteria used for the BLAST-P analyses were: e^{-40}, identity ≥ 50% and length coverage ≥60% of the query sequence. If *O. sativa* gene is yet to be functionally characterized *B. distachyon* gene sequences were used as query and the *O. sativa* gene has been mentioned as no functional characterization (n.f.c). If no homologous sequences were identified within the preset criteria, it has been mentioned as no hit in the table.

VII. Selection and collection of appropriate reproductive and vegetative tissues in *B. tulda* for molecular and gene expression analyses

Diverse vegetative and reproductive tissues from sporadically flowering *B. tulda* plants were collected from Shyamnagar (SHYM7) and Bandel, (SHYM16 and BNDL22), West Bengal, India during April, 2013 to July, 2017. Tissues were snap frozen in liquid nitrogen in the field and then transported to the laboratory and stored in the -80°C freezer for farther experimental usage. Three independent biological replicates were collected for each tissue stages. Vegetative tissues selected for tissue specific expression analyses were young leaf from flowering branch of a flowering culm (YLF), young leaf from non flowering culm (YLN), flag leaf (FL), culm sheath (CS), rhizome (RH), root (R) and internode tissues (I). Three defined reproductive tissues selected for tissue specific expression analyses were early (E), middle (M) and late (L) floral developmental stages. These tissue stages were selected based on the histological observations of the developing floral primordial on inflorescence bud (Biswas et al., 2016)

VIII. Isolation of genomic DNA, total RNA and preparation of cDNA libraries

Young, healthy leaves were snap frozen in liquid nitrogen in the field and then transported to the laboratory and stored in the -80°C freezer. Genomic DNA was isolated from these young, healthy leaves by using DNeasy Plant Mini Kit (Qiagen, Germany). Total RNA was extracted from the selected vegetative and reproductive tissues by using a combination of Trizol (Invitrogen, USA) and RNAeasy Plant Mini Kit (Qiagen, Germany) [Das et al., 2010; 2016]. Total RNA was treated with DNase I (Thermo Scientific, USA) to get rid of any genomic DNA contamination. Quantity was determined in BioSpectrometer (Eppendorf, Germany) and quality of the isolated samples was checked by agarose-formamide gel electrophoresis. Around 1µg total RNA was used for cDNA synthesis using Verso cDNA Synthesis Kit (Thermo Scientific) following manufacturer's protocol. 2µl of $1/20^{th}$ diluted cDNA sample was used for real time RT-qPCR analyses.

IX. Primer designing, PCR amplification, cloning and sequencing of homologous genes

Gene specific degenerate primers were designed by aligning hoologous sequences retrieved from related close monocot genomes. Coding sequences were multiple aligned in MUSCLE

programme and gene specific primers were designed by using Primer3 program. PCR amplification was done using high fidelity Phusion Taq DNA polymerase (Thermo Scientific). Amplified bands of desired molecular weight were eluted from agarose gel by using GeneJET gel elution kit (Thermo Scientific) and cloned into TA vector (pGEM®-T Easy Vector Systems, Promega, USA) or blunt end vector (pJET PCR cloning kit, Thermo Scientific) following the instructions of the manufacturers. Positively transformed colonies were selected on blue-white selection and/or ampicillin medium and plasmids were purified using plasmid isolation kit (GeneJET Plasmid Miniprep Kit, Thermo Scientific). Sequencing was done by Sanger's method, trimmed to get rid of vector sequences, assembled by CAP3 (Huang and Madan, 1999) and were used for all further bioinformatics analyses, sequence comparisons and to obtain remaining sequences of the full length genes or coding sequences.

X. Sequence data and phylogenetic analyses

The amino acid sequences of the identified *B. tulda* genes were aligned with other related sequences using the Clustal W program. The sequences were compared to that of available sequences from related monocot genomes such as *Oryza sativa*, *Phyllostachys meyeri*, *P. heterocycla*, *P. violascens*, *Brachypodium distachyon*, *Sorghum bicolor*, *Hordeum vulgare*, *Zea mays* and *Triticum aestivum*. The phylogenetic tree was constructed by the NJ method with Mega 7 software (Kumar et al., 2016). Bootstrap analysis with values for 1000 replicates was conducted to estimate nodal support. All available literatures were consulted to identify specific amino acid residues within the target genes that are involved in significant biological functions.

XI. *In silico* studies on *Bambusa tulda FLOWERING LOCUS T* gene homologs

Due to the unavailability of crystal structures of BtFT1-4 and sequence/and structure of Bt-14-3-3, interaction between the BtFT-14-3-3 pairs was investigated, keeping 14-3-3 structural coordinates (Taoka et al., 2011) constant from rice Os14-3-3. Homology models of BtFT1-4, which were 86-88% identical to their rice homologue *OsHd3a*, were built using the web version of MODELLER (Sanchez et al., 1998). Interaction analyses were carried out using PyMOL.

XII. Gene expression analyses by real time RT-qPCR method

Gene specific primers were designed from the coding sequences of the targeted genes to measure their transcriptional expression level by real time RT-qPCR analyses. Sequences of four BtFT gene alleles were highly similar to each other and designing individual homolog specific primers was almost impossible. Therefore, one pair of primers was designed for BtFT1 and 2, while another was designed for BtFT3 and 4 and that too were designed only based on one nucleotide sequence divergence at the 3′ end. The identities of the amplified gene products were confirmed by sequencing the amplified PCR products. SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad, USA) was used to measure the expression level of the targeted genes in CFX connect real-time PCR detection system (Bio Rad, USA). The amplification conditions were 30 seconds at 95ºC, 40 cycles of 10 seconds at 94ºC and 40 seconds at 55 or 64ºC. A standard dissociation curve analyses was conducted to confirm the absence of any primer dimers in the amplified products. Data were normalized using

eIF4α as the reference gene and relative fold change in gene expression was estimated following the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

5. Results

I. Morphological characterization of *B. tulda* culm, culm sheath and rhizome

Since flowering in bamboo is very rare, the identification of bamboo is mostly based on the vegetative characters. Important morphological characters of rhizome (underground modified stem), culm (above ground stem) and culm sheath are used as the key identifying characters. Rhizomes studied across populations were sympodial (pachymorph) having short necks. Studies on the fifteen key culm characters (Table 1) and seventeen key culm sheath (Table 2) collected from all the flowering populations (SHYM7, SHYM16 and BNDL22) indicated that the studied plants were *Bambusa tulda*. Furthermore, amplification of Bt609 SCAR marker confirmed their identity as *B. tulda*. Culms were usually straight, bright to dark green in colour and occasionally with vertical yellow striations. Mean height of the culm obtained at different populations ranged from 17.6 ±1.3 to 18.3±1.1 m. Representative culm diameter and length measured at fifth internode ranged from 49.1±5.0 to52.9±3.5 and 364±53.8mm 388±67.0 respectively. Ratio of cavity diameter to culm diameter ranged from 0.40 to 0.48mm. No swollen internodes were observed in any of the populations studied; while nodes are slightly swollen with sheath scars and whitish nodal rings.

Size of the culm-sheaths were variable at different culm heights; adaxial surface glabrous; abaxial surface covered scanty to profuse, black hairs .Total length to breadth of culm sheath at the base ranged from 1.22±0.08 to 1.28±0 mm. Blade was straight in all populations but shape varied from acuminate to ovate. Ratio of the total length to blade length ranged from 2.41±0.89 to 3.50±0.23 mm. Auricles are conspicuous, continuous with blade, unequal, fringed; ligule with serrate margin at all populations.

II. Morphological characterization of *B. tulda* inflorescence, florets and floral organs

In general bamboo inflorescence is broadly similar to that of rice but there exists a few characteristic differences with respect to the position and organization of the inflorescences and also the florets. Inflorescence ranged from a true spikelet on tiller arising from the lowest node or a leafless panicle (pseudo spikelet) with a branching pattern similar to that of a vegetative culm. In bamboo a single branch may bear multiple inflorescences, each of which is subtended by an individual flag leaf (FL). With progression of flowering several inflorescences develop in a basipetal manner where young bamboo inflorescence remains invisible and covered by the leaf sheath of the FL. Rachis of panicle is smooth having 3 to numerous spikelets in capitate-like clusters located in the nodes. True spikelets subtended by shining, chaffy bracts, distichous, 17-50 mm long. The lowermost 2 or 4 florets reduced to empty glumes, followed by 4 to14 fertile florets. Florets are bisexual in nature, 15-19 mm long and 4.5-7 mm broad at the base, lemma broadly acuminate, mucronate, concave, glabrous, many nerved, bright green when fresh, overlapping with the palea. Palea membranous, penicillate, 2-keeled, 5-7 nerved, 9-14 mm long, 3-5 mm broad at the base and ciliae at top. Lodicules 3; pale green to waxy in colour, cuneate, oblong, hyaline, fleshy, 1.5-2.5 mm long, whitish fimbriate at apex. Stamen 6; anthers emarginate, basifixed, purple or

yellow at maturity; pore like linear dehiscence at apex. Filaments thread-like, 10–17 mm during anthesis. Ovary pear shaped at anthesis, 2-2.6 mm long; style 2–3 mm long, hairy; stigmas 3 with Stigmatic papillae, 2–3 mm long, whitish, wavy.

III. Histological analyses to study floral developmental progression

Histological analyses of inflorescence bud at different flowering stages of *B. tulda* plants were done to obtain a clearer insight regarding organization of floret in a spikelet and also the organization of the individual organs. The histological characters were compared with the external morphology of buds. The external morphological features of nodal vegetative buds were indistinguishable from those of early staged inflorescence bud. However the early staged inflorescence buds were slightly smaller in size, pale yellow in colour, and bulged in the middle. Shoot apical meristem of the nodal vegetative bud is dome shaped and covered with compactly arranged leaf primordia. Whereas the early staged inflorescence meristem is slightly smaller in size and triangular in shape. The middle staged floral bud was elongated and bright green in colour. Histological analysis revealed that it is composed of one or two floral primordia at the base of the rachis and an undifferentiated inflorescence meristem at the apex. The late inflorescence buds were long and slender, composed of three to four visible florets having differentiated anther primordia at the base of the rachis and an undifferentiated apical inflorescence meristem.

IV. Morphological and biochemical characterization of pollen grains

The pollen grains were globose, mono-ulcerate with psilate ornamentation ranging from 22.37 to 43.2 mm in diameter. Assessment of pollen viability using KI-I_2 solution and acetocarmine stain revealed a high percentage of non-specific staining, which was obvious by obtaining >95% pollen fertility in *B. tulda* and rice. The tetrazolium test was more specific and 23.97±4.47 to 25.35±1.87 % *B. tulda* pollens were found to be viable while 70.75±5.75% pollen viability was obtained for rice (Table 3).

V. *In vitro* pollen germination assay reveals low pollen germination rate for *B. tulda*

The pollen grains of *B. tulda* germinated best in standard Brewbaker and Kwack's medium containing 25-30% sucrose. Pollen tubes at lower concentration of sucrose were erupted due to osmotic pressure. No germination was observed if sucrose alone was used as the medium. Maximum germination rate of 11.88±2.76% were recorded for *B. tulda* pollens, whereas rice pollen germinated at the rate of 57.07±9.46 % (Table 4).

VI. Bioinformatics identification of important flowering pathway gene homologs in temperate bamboo *Phyllostachys edulis*

Important markers genes related to all the important flowering pathways such as photoperiodic, vernalization, hormonal, and autonomous were selected for this study. Involvement of additional factors such as drought stress and physiological maturity genes were also tested. The BLASTP analyses have identified one or more *P. heterocycla* homologs for the majority of these genes (Table 5). Bamboos are highly polyploidized in nature and consequently the majority of queried genes have been identified in multiple copies. The

majority of these genes were highly sequence wise similar. Examples of this are *FT* and *TFL1* genes, which are members of the Phosphatidylethanolamine-binding protein (PEBP) family and share high sequence similarity (>60%). However, they are functionally antagonistic to each other. Our BLAST analyses have identified five *P. heterocycla* homologs each for *FT* and *TFL1*, which are completely overlapping with each other. Further the BLAST analyses, could not detected *MADS37* or *MADS51* homologs in *P. heterocycla* at the set criterion of e^{-40}, identity ≥50% and length coverage ≥60% of the query sequence (Table 5).

VII. Molecular identification, sequence characterization, phylogenetic analyses of the four BtFT genes

Flowering locus T (FT) is one of the most important floral pathway integrator gene. Four alleles of BtFT genes were identified (BtFT1: KT003820, BtFT2: KT003821, BtFT3: KU726232, BtFT4: KX290774). Homology search of the BtFT1, 2, 3 and 4 sequences identified very high similarity (92-94%) with another bamboo *P. meyeri* FT sequences. Phylogenetic analyses resulted into two different clades of four BtFT sequences. Based on the sequence similarities BtFT1 clustered with BtFT2, while BtFT3 clustered with BtFT4. This result was also supported by the predicted exon-intron organization of the four genes. Four exons and three introns are common for all the four BtFT genes. While the fourth exon was the longest (233 bp), third exon was the shortest in length (41 bp). Exon lengths were highly conserved among the 4 BtFT homologs, however the length of intron 1 was longer in BtFT1, 2 than that of BtFT3, 4. Predicted length of the each BtFT proteins is 178 amino acids containing functional PEBP domain having seven conserved amino acids and two C-terminal amino acid stretches, essential for maintaining the floral inducing function. Detailed sequence analysis confirmed the identity of these 4 genes as FT since important amino acids such as Tyr85 and Gln140, which are essential for FT like function, are present in all the identified B. tulda FT sequences.

VIII. *In silico* studies on the molecular interactions between individual BtFT and Os14-3-3 proteins

The rice FT homologue OsHd3a interacts with Os14-3-3 proteins at the shoot apical meristem (SAM) to form the Hd3a-14-3-3 complex, which is translocated to the nucleus to interact with rice OsFD1, a bZIP transcription factor. The resulting "florigen activation complex" (FAC), promote the conversion of SAM to inflorescence meristem. Seven conserved amino acids located within the PEBP domain of FT contributes to the direct interaction between FT and 14-3-3. Each of these amino acids was individually checked in the four BtFT sequences. Interestingly, two amino acid changes, i.e., Phe101 to Ile101 in BtFT1, 2 and Phe64 to Leu64 in BtFT3, 4 were observed. Therefore, *in silico* protein-protein interaction analyses were conducted to understand the overall interaction efficiency between individual BtFT and 14-3-3 sequences and to determine whether these changes may affect the said interaction or not. Since no crystal structures were available for BtFT proteins and no sequence/and structure of Bt14-3-3, the interaction between BtFT and Os14-3-3 pairs were investigated. To begin with, homology models of BtFT1, 2, 3 and 4 were developed, which were 86-88% identical to their rice homologue OsHd3a. From the 3D models it appeared that while BtFT1 and 2 were almost identical, with no rms deviation, BtFT3 and 4 differed very

slightly. Given the profound homologies among all BtFT alleles, their interaction with Os14-3-3 remained mostly conserved. Surface analysis showed that the interaction interface remained interdigitated, indicating that the BtFT alleles are capable of interacting with 14-3-3 with an almost conserved pattern. Similar to OsHd3a-Os14-3-3 interaction, BtFT1, 2, 3, 4 and Os14-3-3 interaction interface consisted of hydrophobic cavity as well as an in-between acidic lobe (Asp208 and Glu212 of 14-3-3), interacting with Arg130 and Arg62 of BtFT1, 2, 3 and 4 through conserved salt-bridge interactions. These interactions are essential not only for FT's binding with 14-3-3 but also with FD. Multiple sequence alignment revealed subtle differences in BtFT1, 2 and BtFT3, 4 sequences, especially along the hydrophobic cavity. In contrast to OsHd3a sequence (Phe66 and Phe103), Leu64 was present in BtFT3, 4 and Ile101 was present in BtFT1, 2, respectively. In BtFT1, 2, Phe64 stabilized hydrophobic interaction with Ile204 of Os14-3-3, similar to OsHd3a interaction. In BtFT1, 2, Ile101 though made hydrophobic contact with Phe200 of Os14-3-3, in BtFT3, 4, there was a possibility of stacking interaction between Phe101 and Phe200 of Os14-3-3, similar to Hd3a. Though the hydrophobic interactions were subtly different in BtFT1, 2 and BtFT3, 4 compared to OsHd3a-Os14-3-3 interactions, such changes might influence the specificity of BtFT interactions with 14-3-3.

VII. Molecular identification, sequence characterization, phylogenetic analyses of the two BtFD genes

FD belongs to the bZIP transcription factor proteins family. It is a member of the 'FAC' complex, which interacts with the promoter of *APETALA 1* to up regulate it and promote the conversion of SAM to inflorescence meristem. Two alleles of *BtFD* genes were identified (*BtFD1*: MF983712, and *BtFD2*). Homology search of the *BtFD1* sequences identified highest similarity (77%) with another bamboo *Sasa veitchii FD* gene, while the *BtFD2* sequences reported highest similarity (85-87%) with *Panicum hallii* and *Setaria italica* FD proteins. Phylogenetic analyses with related monocot plants resulted into two different clades. Based on the sequence similarities *BtFD1* clustered with *FD1* homologs of other plants, whereas *BtFD2* clustered with other *FD2* genes. There also exists distinct exon-intron organizational difference between BtFD1 and FD2 genes. While *BtFD1* contained two exons and one intron, *BtFD2* contained three exons and two introns. The first exon of *BtFD1* was the longest (537 bp) and the second exon was the shortest in length (72 bp) and the length of the intron was 144bp. Predicted length the BtFD1 protein is 202 amino acids. Both the FD proteins contain a functional bZIP domain. BtFD1 had a conserved SAP domain and flowering specific LSL amino acids sequence, which suggest their possible involvement in flowering. In contrary BtFD2 lack these characteristic sequences and are involved in leaf development in rice.

VIII. Tissue specific expression analyses of BtFT and BtFD genes

The transcriptional expression patterns of the floral pathway integrator (*BtFT1*, *BtFT2*, *BtFT3* and *BtFT4*) and *BtFD1* genes were investigated in ten selected flowering and non-flowering tissue stages to understand their possible role in flowering. Higher transcript abundance of all these genes was detected in young leaves isolated from the flowering culm (YLF) than that of

the non-flowering culm (YLN). However, when the expression levels were compared among ten tissues, the highest expression of BtFT genes were observed in young culm sheath CS. Differences in expression patterns between flowering and non-flowering tissue stages were not observed for the two groups of BtFT genes that were suggested by the phylogenetic analysis. However, in case of *BtFT3*, *4* the expression was also quite high in YLF, which was not the case for *BtFT1*, *2*. Interestingly the highest expression of *BtFD1*gene was observed in YLF.

6. Concluding remarks

Through extensive population level studies on *B. tulda* plants it has been observed that majority of the known, species specific, morphological characters could be observed across populations, although there were a few new characters, which have not been observed before. One such observed key variation was the shape of the culm sheath blade. Usually, the *B. tulda* blade was known to be ovate, but acuminate type of blades was also observed in this study.

This study also identified a number of important reproductive characters. Two different types of inflorescences were observed in *B. tulda*. Inflorescence ranged from a true spikelet to leafless panicle or pseudo spikelet. Also a very low seed setting was observed, which was consistent with the previous study. The current study revealed that- low pollen viability, narrow apical suture causing limited pollen liberation and different developmental rates of androecium and gynoecium (protrandry) could be the possible factors behind low seed setting as a result of sporadic flowering in bamboo.

The homologs for the majority of the flowering genes related to important pathways were identified in the draft, temperate, bamboo *P. heterocycla*. Detailed characterization of *B. tulda Flowering Locus T* homologs revealed that possibly the four homologs are functionally redundant to each other. However, functional divergence with respect to involvement in flowering between the two *B. tulda FD* genes are likely.

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Table 1. Comparison of the 15 Key culm characters of three bamboo populations (SHYM 7, SHYM7 and BNDL22) with 25 known bamboo species (OTUs) used to calculate phylogenetic relationships and identification. Characters included mean height (m), diameter (mm), length of fifth internode (mm), ratio of cavity diameter to total culm diameter, internode bending, colour (yellow with striation = 0, yellow-green = 1, gray-green = 2, pale green = 3, bright green = 4, glossy green = 5, dark green = 6), swollen node, nodal ring (absent = 0, whitish = 1, grayish = 2), nodal sheath scar, hairs at nodal ring, branches come out piercing culm sheath, curved lower nodal branches, diverent culm leaf and branch leaf size, modification of branches (none = 0, thorns = 1, spines = 2), striation on culm.

No	OTUs	Height	Diameter	Internode	Cavity	Bending	Colour	Swollen node	Nodal ring	Sheath scar	Hairs	Branching	Curved branches	Leaf size	Modification	Striation
1	*Arundinaria maling*	4.2±0.8	50±2.0	110±15	0.33±0.05	0	1	1	1	1	1	0	0	0	0	0
2	*Bambusa affinis*	6.5±0.9	40.0±6.0	500±75.0	0.37±0.06	0	2	0	1	1	0	0	0	1	0	1
3	*B. atra*	7.0±1.0	35.0±5.2	600±90.0	0.42±0.06	0	6	0	1	1	0	0	0	0	0	0
4	*B. auriculata*	18.3±2.7	45.0±6.7	400±60.0	0.22±0.03	0	3	0	2	1	1	1	0	1	0	0
5	*B. balcooa*	20.0±2.9	90.0±13.5	200±30.0	0.33±0.05	0	4	1	1	1	1	1	0	1	2	0
6	*B. bambos var. giganteus*	25.0±5.0	110.0±20.0	300±10.0	0.45±0.05	0	2	1	0	0	0	0	1	1	1	0
7	*B. bambos*	15.8±2.5	55.0±8.8	230±36.6	0.36±0.06	0	2	1	0	0	0	0	1	1	1	0
8	*B. burmanica*	15.5±2.2	70.0±5.5	300±30.5	0.52±0.05	0	5	1	1	1	1	0	0	0	0	0
9	*B. multiplex 'riviereorum'*	3.0±0.7	20.0±4.6	200±45.8	0.12±0.03	0	3	1	2	0	0	0	0	0	0	0
10	*B. multiplex 'variagata'*	2.5±0.05	15.0±3.6	180±45.8	0.12±0.03	0	3	1	2	0	0	0	0	0	0	0
11	*B. Nutans*	12.5±3.2	60.0±8.7	400±65.8	0.4±0.07	0	4	0	1	1	0	1	0	1	0	1
12	*B. oliveriana*	14.0±1.5	37.5±4.1	350±38.4	0.53±0.06	0	5	1	1	1	1	0	0	0	0	0
13	*B. polymorpha*	20.2±3.2	100.0±8.7	500±65.8	0.45±0.07	0	1	0	1	1	0	1	0	1	0	1
14	*B. striata*	16.5±0.9	70.0±9.4	190±18.6	0.42±0.06	0	0	1	2	1	0	0	0	0	0	1
15	*B. teres*	15.5±3.2	70.0±8.7	500±65.8	0.48±0.07	0	6	0	1	1	0	1	0	1	0	1
16	*B. tulda*	18.3±3.2	50.0±8.7	380±65.8	0.4±0.07	0	6	0	1	1	0	1	0	1	0	1
17	*B. vulgaris*	19.8±2.2	80.0±8.9	230±25.5	0.5±0.06	0	4	1	2	1	0	0	0	0	0	1
18	*B. wamin*	7.0±1.0	120.0±17.0	120±18.6	0.33±0.05	0	6	1	1	1	0	0	0	0	0	0
19	*Dendrocalamus giganteus*	30.5±3.2	130.0±13.4	330±34.2	0.69±0.07	0	2	0	2	1	1	0	0	1	0	0
20	*D. strictus*	14.0±2.7	50.0±9.5	200±71.4	0.6±0.11	0	1	1	0	1	0	0	1	0	0	0

21	*Gigantochloa atroviolacea*	21.0±2.8	60.0±8.0	375±26.4	0.54±0.07	0	6	0	0	0	0	0	0	1	0	0
22	*Melocanna baccifera*	15.0±1.2	55.0±5.0	220±15.0	0.32±0.05	0	4	0	0	0	0	0	0	0	0	0
23	*Oxytenanthera abyssinica*	8.0±1.3	80.0±5.0	180±15.0	0.55±0.06	0	4	0	1	1	0	0	0	1	0	0
24	*Pseudobambusa kurzii*	9.14±1.8	35.0±7.0	220±43.9	0.5±0.09	1	2	0	1	1	0	0	0	0	0	0
25	*Thamnocalamus spathiflorus subsp. spathiflorus*	5.0±1.5	15.0±5.0	120±20.0	0.33±0.05	0	1	1	1	1	1	0	0	0	0	0
26	SHYM7	18.1±1.8	52.9±3.5	364±53.8	0.40±0.02	0	6	0	1	1	0	1	0	1	0	1
27	SHYM16	17.6±1.3	49.1±5.0	377±34.3	0.40±0.05	0	6	0	1	1	0	1	0	1	0	1
28	BNDL22	18.3±1.1	50.8±3.9	388±67.0	0.40±0.02	0	6	0	1	1	0	1	0	1	0	1

Table 2. Comparison of the 17 Key culm-sheath characters of three bamboo populations (SHYM 7, SHYM7 and BNDL22) with 25 known bamboo species (OTUs) used to calculate phylogenetic relationships and identification. Characters included ratio of total length to breadth at base, ciliate margin, pubescent adaxial side, pubescent abaxial side, hair colour (none = 0, golden brown = 1, brown = 2, dark brown = 3, black = 4), number of hairs (absent = 0, scanty = 1, profuse = 2), ratio of total length versus blade length, shape of blade (triangular = 0, acuminate = 1, lanceolate = 2, ovate = 3), blade reflexed, hairy margin of blade, ligule margin (entire = 0, dentate = 1, serrate = 2), hairs on ligule, auricle, auricle continuous with blade, bristles on auricle, auricle fringed, variable culm sheath size at diverent culm height.

No	Species	Length / Breadth	Margin	Pub. Adax	Pub. abax	Hair colour	Hair number	Total / blade	Shape	Blade reflexed	Hairy margin	Ligule margin	Hairs on ligule	Auricle	Auricle cont.	Bristles	Auricle fringed	Sheath size
1	*Arundinaria maling*	3.0±0.85	0	0	0	0	0	4.2±0.55	1	1	1	2	1	1	0	1	0	0
2	*Bambusa affinis*	1.55±0.23	0	0	1	4	1	4.43±0.66	3	1	1	0	0	0	0	0	0	0
3	*B. atra*	3.33±0.49	0	0	1	1	1	2.0±0.29	3	0	0	0	1	1	1	1	0	0
4	*B. auriculata*	1.5±0.22	1	0	1	4	2	2.45±0.37	3	0	1	1	1	1	1	1	1	1
5	*B. balcooa*	1.59±0.24	1	0	1	4	1	4.15±0.64	0	0	0	2	1	0	0	0	0	0
6	*B. bambos*	2.8±0.37	1	1	0	4	1	3.66±0.62	1	0	0	1	1	1	1	1	1	0

	var. giganteus																	
7	*B. bambos*	2.92±0.47	1	1	0	4	1	3.88±0.62	1	0	0	1	1	1	1	1	1	0
8	*B. burmanica*	2.60±0.30	0	0	1	4	1	2.43±0.26	1	0	0	2	0	1	0	1	0	0
9	*B.multiplex 'riviereorum*	2.87±0.66	0	0	0	0	0	2.87±0.56	2	0	0	0	0	1	0	1	0	0
10	*B. Multiplex 'variagata',*	2.75±0.55	0	0	0	0	0	2.77±0.55	2	0	0	0	0	1	0	1	0	0
11	*B. nutans*	1.15±0.18	0	0	1	4	2	3.42±0.64	3	0	0	2	0	1	1	1	1	1
12	*B. oliveriana*	2.69±0.30	0	0	1	4	1	2.33±0.26	1	0	0	2	0	1	0	1	0	0
13	*B. polymorpha*	1.20±0.18	0	0	1	4	2	3.50±0.64	3	1	0	2	0	1	1	1	1	1
14	*B. striata*	1.78±0.22	0	0	1	3	2	2.5±0.31	3	0	0	2	1	1	0	0	0	0
15	*B. teres*	1.25±0.18	0	0	0	4	2	3.20±0.64	3	0	0	2	0	1	1	1	1	1
16	*B. tulda*	1.04±0.18	0	0	1	4	2	3.67±0.64	3	0	0	2	0	1	1	1	1	1
17	*B. vulgaris*	1.65±0.15	1	0	1	3	2	2.75±0.30	1	0	0	0	1	1	0	1	0	1
18	*B. wamin*	0.87±0.13	1	0	1	2	2	2.08±0.31	3	0	1	2	1	1	0	0	0	0
19	*Dendrocalamus giganteus*	1.3±0.13	0	0	1	2	2	3.36±0.35	2	1	0	2	1	0	0	0	0	1
20	*D. strictus*	1.39±0.26	1	0	1	1	2	3.85±0.73	0	0	1	2	1	1	1	0	0	0
21	*Gigantochloa atroviolacea*	1.31±0.17	0	0	1	1	1	3.0±0.39	2	1	0	0	1	0	0	0	0	1
22	*Melocanna baccifera*	1.16±0.15	0	0	1	1	1	1.25±0.42	2	0	0	1	0	1	1	1	1	0
23	*Oxytenanthera abyssinica*	2.1±0.25	1	0	1	3	1	1.55±0.22	2	0	1	0	0	0	0	0	0	0
24	*Pseudobambusa kurzii*	3.37±0.67	1	0	1	4	2	1.8±0.36	2	1	1	1	1	1	1	0	0	1
25	*Thamnocalamus spathiflorus subsp. spathiflorus*	3.6±0.85	0	0	0	0	0	3.6±0.55	1	1	1	2	1	0	0	0	0	0
26	SHYM7	1.28±0.16	0	0	1	3	1	2.41±0.89	3	0	0	2	0	1	1	1	1	1
27	SHYM16	1.22±0.08	0	0	1	3	1	3.44±0.28	3	0	0	2	0	1	1	1	1	1
28	BNDL22	1.22±0.23	0	0	1	3	1	3.50±0.23	3	0	0	2	0	1	1	1	1	1

Table 3. Comparison of *Bambusa tulda* pollen viability across population. Pollens from different population treated with 0.5% tetrazolium (TTC) dissolved in 15% sucrose solution. Samples were incubated for 30 mins at 37°c and observed under microscope. Red coloured pollen were counted as viable and the colourless one as non viable. Percentage of pollen viability was calculated by dividing the number of stained pollens by the total number of pollen grains per field of view and expressed in percentage values.

B tulda population	Replicate	Microscopic Field	No. of dark red stained pollen	No. of light red stained pollen	Total no. of stained pollen	No. of non stained pollen	Total no. of pollen	Pollen viability (%)
SHYM16	1	1	8	9	17	45	62	25.35±1.87
		2	16	15	31	95	126	
		3	9	11	20	71	91	
	2	1	7	10	17	43	60	
		2	11	13	24	69	93	
		3	9	12	21	59	80	
	3	1	21	6	27	82	109	
		2	14	11	25	76	101	
		3	15	10	25	78	103	
BNDL22	1	1	12	9	21	109	130	23.94±4.28
		2	15	14	29	136	165	
		3	14	15	29	82	111	
	2	1	8	10	18	55	73	
		2	19	11	30	72	102	
		3	7	12	19	55	74	
	3	1	5	9	14	39	53	
		2	17	12	29	88	117	
		3	10	8	18	55	73	
SHYM7	1	1	9	8	17	44	61	25.81±1.66
		2	7	12	19	48	67	
		3	15	12	27	78	105	
	2	1	9	3	12	35	47	
		2	5	11	16	47	63	
		3	19	13	32	107	139	
	3	1	15	5	20	63	83	
		2	9	12	21	59	80	
		3	17	7	24	68	92	

Table 4. Comparison of *in vitro* pollen germination rate of *Bambusa tulda* across population. Freshly collected pollen grains dusted in standard Brewbaker and Kwack's medium and incubated for 30 mins before microscopic observation. Pollen grains were considered as germinated if the length of pollen tube was greater than the diameter of the pollen grain. Germination percentage was determined by dividing the number of germinated pollen grains per field of view by the total number of pollen per field.

B tulda population	Replicate	Microscopic Field	No. of germinated pollen	No. of total pollen	Germination (%)
SHYM16	1	1	3	20	13.48±4.05
		2	2	15	
		3	1	11	
	2	1	4	23	
		2	2	18	
		3	3	15	
	3	1	2	16	
		2	3	19	
		3	1	14	
BNDL22	1	1	1	16	15.16±5.94
		2	2	15	
		3	2	8	
	2	1	2	11	
		2	1	8	
		3	3	23	
	3	1	3	17	
		2	3	14	
		3	1	11	
SHYM7	1	1	2	13	14.35±2.34
		2	3	18	
		3	3	19	
	2	1	2	12	
		2	1	7	
		3	3	20	
	3	1	1	9	
		2	1	10	
		3	2	14	

Table 5. Identification of important flowering gene homologs in the model temperate grass- *Brachypodium distachyon* and temperate bamboo- *Phyllostachys heterocycla.* Amino acid sequences of *Oryza sativa* was used as query in BLAST-P analyses. The criteria used for this analysis were e^{-40}, identity >55%, length coverage> 60% of the query sequence. In the absence of any functional characterization of a gene in *O. sativa* (no functional characterization, n.f.c), gene sequences obtained from *B. distachyon* were used as query sequences. When no homologous sequences were identified in our set criteria, it is mentioned as no hit. **Hd3a and RFT1 are sequentially similar and also functionally redundant to each other in *O. sativa*. Therefore, many of the identified homologs of these two genes are overlapping within individual species studied.

Flowering pathways/ regulators	Genes	*O. sativa* identifiers used as query	BLAST hits in *B. distachyon*	BLAST hits in *P. heterocycle*
Vernalization	*VRN1*	*Os03g54160*	*Bradi1g08340* *Bradi1g59250*	*PH01000606G0250* *PH01000222G1190*
	VIL2	*Os12g34850*	*Bradi4g05950* *Bradi2g36237*	*PH01000006G3670* *PH01000674G0720* *PH01000258G0590* *PH01001556G0190*
	VIL3	*Os02g05840*	*Bradi3g04140* *Bradi1g33450*	*PH01000836G0140* *PH01000114G1300* *PH01002795G0050*
	FLC/MADS37	n.f.c	*Bradi3g41297*	No hit
	FLC/MADS51	*Os01g69850*	*Bradi2g59191*	No hit
		n.f.c	*Bradi2g59119*	No hit
Photoperiod	*PHY A*	*Os03g51030*	*Bradi1g10520* *Bradi1g10510* *Bradi1g08400*	*PH01000222G1330* *PH01000606G0390*
	PHY B	*Os03g19590*	*Bradi1g64360* *Bradi1g08400*	*PH01000013G2240* *PH01000013G2230* *PH01000222G1330* *PH01000606G0390*
	CRY 1	*Os02g36380*	*Bradi3g46590* *Bradi5g11990* *Bradi3g49204*	*PH01000349G1020* *PH01000968G0540* *PH01002373G0140* *PH01000263G1210* *PH01002304G0120*
	CRY2	*Os02g41550*	*Bradi3g49204* *Bradi5g11990* *Bradi3g46590*	*PH01000968G0540* *PH01000349G1020* *PH01002304G0120* *PH01002373G0140* *PH01002304G0180*
	CCA1	*Os08g06110*	*Bradi3g16515*	*PH01001283G0510* *PH01000383G0300*

	ELF 3	*Os01g38530*	*Bradi2g14290*	*PH01000391G0450* *PH01000410G0960*
	ELF 4	*Os11g40610*	*Bradi4g13227* *Bradi1g60090*	*PH01002557G0050*
	TOC 1	*Os02g40510*	*Bradi3g48880*	*PH01003618G0130* *PH01000345G0790*
	COP 1	*Os02g53140*	*Bradi3g57667*	*PH01000928G0310* *PH01000311G0870*
	FKF 1	*Os11g34460*	*Bradi4g16630* *Bradi1g33610* *Bradi3g04040*	*PH01002958G0010* *PH01000114G1110* *PH01000836G0340* *PH01002213G0250* *PH01007024G0030*
	ZTL	*Os06g47890*	*Bradi1g33610* *Bradi3g04040* *Bradi4g16630*	*PH01007024G0030* *PH01002213G0250* *PH01000836G0340* *PH01000114G1110* *PH01002958G0010*
	CO	*Os06g16370*	*Bradi1g43670* *Bradi3g56260*	*PH01005551G0030*
	GI	*Os01g08700*	*Bradi2g05226*	*PH01002142G0290* *PH01001722G0270*
Autonomous	*FCA*	*Os09g03610*	*Bradi4g08727*	*PH01002230G0270*
	FY	*Os01g72220*	*Bradi2g60817*	*PH01001355G0380* *PH01002367G0110* *PH01002367G0090*
	FLD	*Os04g0560300*	*Bradi5g18210* *Bradi3g58720*	*PH01000272G0440*
	FPA	*Os09g0516300*	*Bradi4g35250*	*PH01000191G0930*
	FVE	*Os01g0710000*	*Bradi2g47940*	*PH01000048G0850* *PH01000241G0710*
	LD	*Os01g70810*	*Bradi2g59937*	*PH01006816G0010*
	FLK	*Os12g40560*	*Bradi4g02690* *Bradi1g14320*	*PH01000025G1210*
Gibberellic acid	*GA1*	*Os02g17780*	*Bradi2g33686*	*PH01000557G0660* *PH01002827G0080* *PH01004049G0170*
	KAO	*Os06g02019*	*Bradi1g51780* *Bradi1g30807* *Bradi5g00467* *Bradi4g05240*	*PH01000083G0900* *PH01003454G0070* *PH01000246G0620*
	GA2ox1	*Os05g06670*	*Bradi2g34837* *Bradi2g12440*	*PH01000685G0370*
	GA2ox2	*Os01g22910*	*Bradi2g12440* *Bradi2g34837*	*PH01000685G0370*

	GA2ox3	*Os01g55240*	*Bradi2g50280* *Bradi2g19900* *Bradi2g16750* *Bradi2g16727* *Bradi2g32577* *Bradi2g06670*	*PH01000018G1890* *PH01001124G0470* *PH01001567G0040* *PH01000273G0650* *PH01000274G0980*
	OsGA3ox1	*Os05g08540*	*Bradi2g04840* *Bradi4g23570*	*PH01002274G0400*
	OsGA3ox2	*Os01g08220*	*Bradi2g04840* *Bradi4g23570*	*PH01002274G0400*
	GID1	*Os05g33730*	*Bradi2g25600*	*PH01001316G0350* *PH01002734G0310*
	GID2	*Os02g36974*	*Bradi3g46950*	No hit
	GAMYB	*Os01g59660*	*Bradi2g53010*	*PH01000009G0060* *PH01000029G1950*
Integrator	*FT*	*Os06g06320/Hd3a***	*Bradi1g48830* *Bradi2g07070* *Bradi5g14010* *Bradi3g48036* *Bradi2g49795* *Bradi1g38150* *Bradi2g19670* *Bradi4g39730* *Bradi4g39760* *Bradi3g08890* *Bradi4g39750* *Bradi4g42400* *Bradi3g44860* *Bradi5g09270* *Bradi1g42510*	*PH01002288G0050* *PH01001134G0390* *PH01003363G0220* *PH01002570G0010*
		*Os06g06300/RFT1***	*Bradi1g48830* *Bradi2g07070* *Bradi3g48036* *Bradi5g14010* *Bradi2g49795* *Bradi2g19670* *Bradi3g08890* *Bradi1g38150* *Bradi4g39730* *Bradi4g39760* *Bradi4g42400* *Bradi4g39750* *Bradi4g35040* *Bradi3g44860* *Bradi5g09270* *Bradi2g27860* *Bradi2g01020*	*PH01002288G0050* *PH01001134G0390* *PH01003363G0220* *PH01002570G0010* *PH01007086G0020*

	SOC1 /MADS50	*Os03g03070*	*Bradi3g32090* *Bradi1g77020* *Bradi3g51800*	*PH01000759G0450* *PH01000059G1270* *PH01000107G0570* *PH01002152G0120*
Drought	*Dof12*	*Os03g07360*	*Bradi1g73710* *Bradi3g25670*	*PH01000113G0300* *PH01000188G0230* *PH01000219G0080* *PH01001264G0440*
Physiological maturity	*LFY*	*Os04g51000*	*Bradi5g20340*	*No hit*
	TFL1	*Os11g05470/RCN1*	*Bradi4g42400* *Bradi5g09270* *Bradi3g44860* *Bradi1g48830* *Bradi2g07070* *Bradi3g48036* *Bradi2g49795* *Bradi5g14010* *Bradi2g19670* *Bradi3g08890* *Bradi2g01020* *Bradi1g38150* *Bradi4g39730*	*PH01001134G0390* *PH01003363G0220* *PH01002570G0010* *PH01007086G0020* *PH01002288G0050*
		Os12g05590/RCN3	*Bradi4g42400* *Bradi5g09270* *Bradi3g44860* *Bradi1g48830* *Bradi2g07070* *Bradi3g48036* *Bradi2g49795* *Bradi5g14010* *Bradi2g19670* *Bradi3g08890* *Bradi2g01020* *Bradi1g38150* *Bradi4g39730*	*PH01001134G0390* *PH01003363G0220* *PH01002570G0010* *PH01007086G0020* *PH01002288G0050*